

**IN THE UNITED STATES PATENTS AND
TRADEMARKS OFFICE**

RECEIVED
01 JUN - 1 AM 10:38

APPLICANT: Bayer AG
SERIAL NO.: 08/925,372
FILED: September 8, 1997
FOR: NON SYSTEMIC CONTROL OF PARASITES
UNIT: 1209
EXAMINER: Allen J. Robinson

DECLARATION

I, Dr. Olaf Hansen of Leverkusen Germany a German citizen hereby declare:

- that I am a veterinarian having studied at the Veterinarian University of Hannover (Germany),
- that in 1995 I received my approbation as veterinarian,
- that I continued my studies and received a doctor degree in December 1996, for my thesis on medical treatment of toxoplasmosis in cats,
- that in September 1996 I started working at Bayer as a post doctoral intern,
- that in September 1998 I became fully employed by Bayer as head of laboratory in Bayer's institute of parasitology,

- that my special field of work is the testing and investigation of active compounds against ectoparasites on animals and especially against fleas, lice, mites and ticks on cats and dogs,
- that the tests of the following report have been run under my surveillance and supervision:

Efficacy of different derivatives of nicotiny-insecticides after spot on and oral application in a dosage of 10 mg/kg body weight against fleas (*Ctenocephalides felis*) on dogs

Identification

Each dog was identified by ear number. The following details were recorded: Ear number, treatment group, treatment day and medication history. All animals used in this study were female beagle dogs and were older than one year.

Location

All dogs were housed in individual cages.

Pretrial Procedures

Only healthy and clinically sound dogs were included in this study. Wild and fractious dogs were excluded.

General husbandry

Routine feeding, watering and cleaning was performed according to current standards of the Animal Health Facilities (SOP 6079).

Feeding and Water

The dogs received commercial feed. Drinking water was available *ad libitum*.

Study Design

The study was divided in three successive trials (see table 1). All trials (A, B and C) were conducted in the same way. At each trial 41 dogs were divided in 7 groups. Six groups consisted of 6 dogs, the untreated control group consisted of 5 dogs (see table 1). The applied volume for each dog is listed in table 3.

Table 1: Overview of Treatment

	Trial	Dogs per group	COMPOUND	FORMULATION	DOSAGE
A	A1	6	Thiamethoxam	Spot on 10 %	10 mg / kg body weight
		6		Oral 10 %	
	A2	6	Dinotefuran	Spot on 10 %	
		6		Oral 10 %	
	A3	6	Nitenpyram	Spot on 10 %	
		6		Oral 10 %	
		5	Control	/	/
B	B1	6	B1	Spot on 10 %	10 mg / kg body weight
		5*		Oral 10 %	
	B2	6	Thiacloprid	Spot on 10 %	
		5*		Oral 10 %	
	B3	6	Acetamiprid	Spot on 10 %	
		5*		Oral 3 %	
		5	Control	/	/
C	C1	6	Imidacloprid	Spot on 10 %	10 mg / kg body weight
		5**		Oral 10 %	
	C2	6	Chlothianidin	Spot on 5 %	
		5**		Oral 2,5 %	
	C3	6	AKD 1022 ^{3N}	Spot on 10 %	
		6		Oral 10 %	
		5	Control	/	/

* 1 dog of the group vomited --> exclusion

**1 dog of the group wild and fractious --> exclusion

At day -4 and day -1 all dogs of each trial were infested with approximately 100 unfed adult cat fleas (*Ctenocephalides felis*) (laboratory strain Monheim). On day 0 each dog was combed for 5 minutes for pretreatment flea counts. Fleas were set back on each dog. After combing all dogs without the five control dogs were treated. Two days after treatment all dogs were combed again for efficacy flea counts. On days 5, 7, 14, 21 and 28 all dogs were infested with approximately 100 unfed adult

cat fleas (laboratory strain Monheim). Each dog was combed 10 days after infestation (days 7, 9, 16, 23, 30) and fleas were removed (see table 2).

Table 2: Overview of Study Design

Trial day	Action
Day 4 (-)	Infestation with approximately 100 unfed adult cat fleas
Day 1 (-)	Infestation with approximately 100 unfed adult cat fleas
Day 0	Combing / Flea counts / Set fleas back on dog / Treatment
Day 2	Combing / Flea counts / Remove fleas
Day 5	Infestation with approximately 100 unfed adult cat fleas
Day 7	Combing / Flea counts / Remove fleas
	Infestation with approximately 100 unfed adult cat fleas
Day 9	Combing / Flea counts / Remove fleas
Day 14	Infestation with approximately 100 unfed adult cat fleas
Day 16	Combing / Flea counts / Remove fleas
Day 21	Infestation with approximately 100 unfed adult cat fleas
Day 23	Combing / Flea counts / Remove fleas
Day 28	Infestation with approximately 100 unfed adult cat fleas
Day 30	Combing / Flea counts / Remove fleas

For the spot-on trials the compounds were used in the form of a 5 % or 10 % m/v formulation in which the active compounds (m in g) were dissolved in a mixture of 16,5 g propylene carbonate, 83,2 g benzylalcohol and 0,1 g butylhydroxytoluene (v in ml).

For the oral trials the compounds were used in the form of a 2,5 %, 3 % or 10 % solution of the active compounds (m in g) in propylene carbonate (v in ml).

Treatment

Table 3: Weight and application

Dog No.	Compound	Formulation	Weight [kg]	Dosage [mg/kg bw]	Dosage [ml]
3131858	Thiamethoxam	Spot on 10%	9.4	10	0.94
3082971			6.7		0.67
3121682			6.3		0.63
3131823			11.2		1.12
3145051			8.8		0.88
3100693			6.0		0.60
3118312		Oral 10%	7.7		0.77
3089398			10.2		1.02
3101088			9.8		0.98
3132137			12.2		1.22
3143139			9.4		0.94
3143732			7.3		0.73
3109488	Nitenpyram	Spot on 10%	10.4	10	1.04
3093875			6.3		0.63
3146111			7.0		0.70
3120881			9.4		0.94
3123146			6.6		0.66
3093522			9.4		0.94
3119122		Oral 10%	9.0		0.90
3125840			8.5		0.85
3084647			10.1		1.01
3145310			8.5		0.85
3100537			6.1		0.61
3131584			7.0		0.70
3092143	Dinotefuran	Spot on 10%	10.3	10	1.03
3095878			7.3		0.73
3084116			9.0		0.90
3099717			10.2		1.02
3100940			5.7		0.57
3081613			10.2		1.02
126412		Oral 10%	9.4		0.94
137597			7.2		0.72
139492			10.3		1.03
105822			7.3		0.73
148637			8.5		0.85
098460			5.9		0.59

Cont. Table 3: Weight and application

Dog No.	Compound	Formulation	Weight [kg]	Dosage [mg/kg bw]	Dosage [ml]
G 331	Acetamiprid	Spot on 10%	11.8	10	1.18
G 421			11.9		1.19
G 291			12.5		1.25
G 319			12.6		1.26
E 409			9.5		0.95
423			9.2		0.92
F 415		Oral 3%	9.4		3.13
G 427			11.8		3.93
G 201			13.1		4.37
F 255			11.3		3.77
F 259			10.5		3.50
G 441	Thiacloprid	Spot on 10%	7.9	10	0.79
G 267			9.0		0.90
E 229			9.4		0.94
E 415			9.0		0.90
F 361			8.6		0.86
G 373			9.4		0.94
F 297		Oral 10%	10.4		1.04
G 223			11.0		1.10
F 253			12.0		1.20
G 277			11.8		1.18
G 433			10.1		1.01
G 355	B1	Spot on 10%	9.8	10	0.98
G 397			8.8		0.88
G 367			8.0		0.80
G 353			9.0		0.90
E 391			12.5		1.25
G 315			11.3		1.13
G 403		Oral 10%	10.7		1.07
G 409			9.7		0.97
F 309			11.7		1.17
G 439			11.0		1.10
221			12.9		1.29
I 295	Imidacloprid	Spot on 10%	10.0	10	1.00
J 345			10.2		1.02
J 407			8.9		0.89
J 375			10.4		1.04
J 245			9.4		0.94
I 309			10.6		1.06
I 287		Oral 10%	11.3		1.13
J 217			12.1		1.21
I 263			13.5		1.35
I 319			11.2		1.12
J 273			12.0		1.20

Cont. Table 3: Weight and application

Dog No.	Compound	Formulation	Weight [kg]	Dosage [mg/kg bw]	Dosage [ml]
J 257	AKD 1022	Spot on 10%	9.5	10	0.95
I 259			9.8		0.98
I 275			9.4		0.94
J 205			10.6		1.06
I 207			9.4		0.94
J 327			8.9		0.89
I 273		Oral 10%	9.8		0.98
G 317			10.7		1.07
J 377			10.4		1.04
J 317			9.2		0.92
I 289			10.7		1.07
I 297			8.9		0.89
I 325	Clothianidin	Spot on 5%	11.4	10	2.28
I 307			12.2		2.44
J 297			13.8		2.76
J 305			12.6		2.52
J 269			7.8		1.56
J 207			8.8		1.76
J 237		Oral 2.5%	13.2		5.28
I 261			8.0		3.20
I 303			9.1		3.64
I 315			9.5		3.80
G 351			12.0		4.80

Route and Method of Administration

All nine nicotinyl-derivatives were applied as a spot on and as an oral formulation in a dosage of 10 mg / kg body weight.

Immediately before treatment the investigator confirmed the animal's identification. The required volume of compound was applied at one spot on the skin between the shoulder blades or as an oral formulation directly in the mouth via capsules.

Method of Parasite Infestation

Fleas were transferred from the breeding containers to glass vials with each vial containing approximately 100 fleas. The fleas were seeded directly onto the individual dogs.

Assessment Methods

Each dog's identity was verified prior to treatment, counts or infestation. A different comb was used for each group of dogs. The combs were for sale (Flohkamm Herberholz, Hauptner, article number: 68491).

Combing Method

Life fleas were counted by combing the dogs in the following order:

1. Outside hind legs
2. Tail and anal areas
3. Lateral area up to and including the shoulder blades
4. Abdominal area from chest to inside hind legs
5. Fore legs
6. Dorsal strip from base of tail to shoulder blades
7. All neck and head areas
8. Spot on area was excluded from combing

Fleas showing no movement were considered as dead.

Combing time was restricted to 5 minutes per dog (Zakson et al. 1995). With the exception of day 0 fleas which had been combed out were not set back on the dog.

Efficacy Calculation

For assessment of efficacy the modified Abbott's Formula was used:

$$\text{Efficacy} = \frac{\left\{ \begin{array}{c} \text{average number of fleas} \\ \text{of control group} \end{array} \right\} - \left\{ \begin{array}{c} \text{average number of fleas} \\ \text{of Treatment Group} \end{array} \right\}}{\text{average number of fleas of control group}} \times 100$$

Efficacy of different chloronicotinylenes after spot on and oral application in a dosage of 10 mg/kg body weight against fleas (*Ctenocephalides felis*) on dogs

Compound	Trial	Application	Efficacy (%) after					
			2 ^d	7 ^d	9 ^d	16 ^d	23 ^d	30 ^d
(Thiamethoxam)	A	Spot-on	100	100	100	100	100	100
		Oral	100	63.8	26.9	9.7	0	0
(Dinotefuran)	A	Spot-on	100	100	100	100	98.4	84.2
		Oral	100	61.1	26.2	0	21.5	0
(Nitenpyram)	A	Spot-on	95.8	99.5	97.8	93.1	88.7	74.1
		Oral	98.6	37.3	0	0	0	0
(B1)	B	Spot-on	100	100	100	100	99.0	99.1
		Oral	100	33.7	12.7	10.1	0	19.1
(Thiacloprid)	B	Spot-on	46.7	95.6	98.8	100	81.4	58.8
		Oral	72.0	31.6	0	11.4	0	19.1
(Acetamiprid)	B	Spot-on	73.3	98.2	98.8	96.8	94.1	61.6
		Oral	96.0	35.8	0	2.5	0	2.2
(IMIDACLOPRID)	C	Spot-on	100	100	100	100	96.6	91.2
		Oral	100	13.4	10.8	5.2	33.0	22.8
(Chlothianidin)	C	Spot-on	100	100	100	100	100	100
		Oral	100	0	0	0	0	0
(AKD 1022)	C	Spot-on	100	100	100	100	96.6	96.6
		Oral	100	0	13.9	2.1	0	0

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Leverkusen, Germany, this 15th day of February


